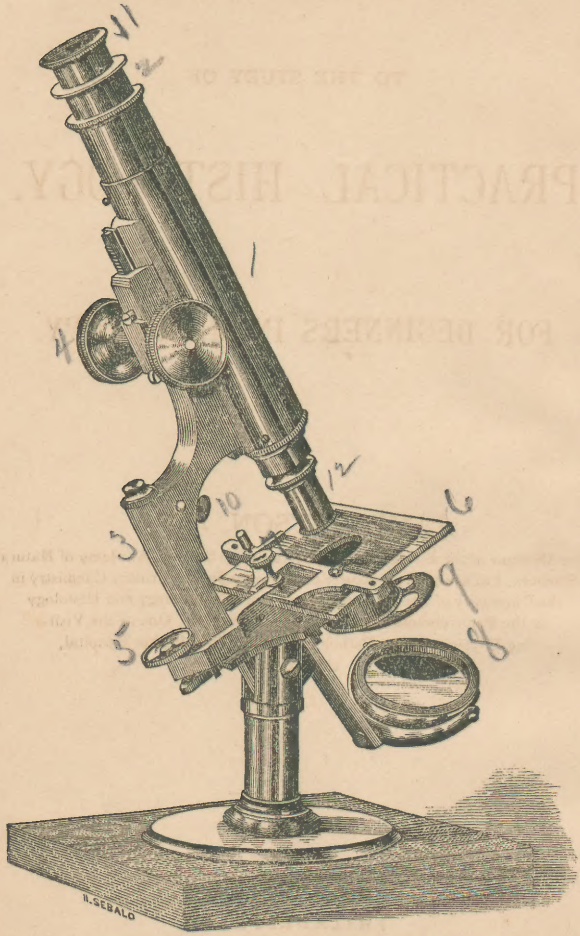
INTRODUCTION
TO
PRACTICAL HISTOLOGY.

TYSON.



ZENTMAYER'S NEW STUDENT'S MICROSCOPE—⅓ ACTUAL SIZE.

AN INTRODUCTION

TO THE STUDY OF

PRACTICAL HISTOLOGY.

FOR BEGINNERS IN MICROSCOPY.

BY

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THE substance of this essay was read before the Biological and Microscopical Section of the Academy of Natural Sciences, and was a simple narrative of a short personal experience in the laboratory of Dr. Klein, of London, and that of Prof. Stricker, of Vienna. First published in the *Philadelphia Medical Times*, a few extra copies were reprinted for my friends. So many of these were sought that it seemed desirable to issue an edition for the use of my own classes and such others as may wish the book. Accordingly, I have rearranged the matter, omitted some portions having a less practical bearing, and added others hoped to be more useful,—such as short sections on the selection of a microscope, measuring and drawing objects, hæmatoxylin staining, the study of nervous tissues, of bone, etc.,—striving to make the little work what its title-page announces, an *introduction* to the study of practical histology.

It does not pretend to be complete; nor is it intended to substitute the admirable works of Beale, Frey, or Klein in Burdon-Sanderson's Hand-Book for the Physiological Laboratory; but rather, by opening the way, to invite to their use as more advanced guides.

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AN INTRODUCTION TO PRACTICAL HISTOLOGY.

SELECTION OF A MICROSCOPE.

IN choosing an instrument, simplicity of construction is the first desideratum. A microscope too large and unwieldy, or composed of too many parts, is less likely to be often used than one which is easily portable and always ready. For the medical student and practitioner there should be *two object-glasses*, an $\frac{8}{10}$ or 1 inch, according to the English and American system, as a lower power, and a $\frac{1}{5}$ or $\frac{1}{4}$ inch for a higher; and there is great advantage, especially to the beginner, in having a *double nose-piece*, so that one objective may be rapidly substituted for another. There should also be *two eye-pieces*, a lower and a higher,—an A and B, or 1 and 2. With such a combination, a range of amplification from 50 to 400 diameters is obtained. According to the French and German system, the objectives above referred to would be indicated by Nos. 1 and 5. If a higher power is desired, it should be an immersion lens, an English or American $\frac{1}{10}$ inch or German No. 9 or 10. A *bull's-eye condenser*, *stage micrometer*, and *camera lucida*, or other apparatus for drawing, are also indispensable in a student's microscope.

The *tube* of the instrument, though it should be capable of further elongation, *should not be so long*

that the microscope cannot be used in the vertical position unless the observer is standing or using an inconveniently high chair. This is a matter of no little importance, since the instances are rapidly increasing in which the microscope can only be used in the vertical position, though it should also be provided with a joint so as to admit of inclination when this is desired. The new student's microscope of Mr. Joseph Zentmayer, figured in the frontispiece, has been constructed with this convenient length of tube as one of its objects, and is the only American instrument with which I am familiar which can be conveniently used in the upright position. Its cost, including double nose-piece, stage micrometer, camera lucida, and condenser, is $112; with Wenham's binocular attachment and four eye-pieces, $140. Of the continental stands, the well-known and highly-esteemed Hartnack's and Nachet's correspond to this, and cost, including duty, nearly as much. H. Crouch, of London, produces a stand after the Hartnack pattern.

The micrometer screw or fine adjuster of the microscope should be of good construction, and although for the coarse adjuster a rack movement and milled head are not indispensable, they are very convenient and desirable. The lower-priced continental stands are not usually provided with them.

ESTIMATION OF MAGNIFYING POWER.

The first step after obtaining a microscope is to calculate its magnifying power. This is a very simple process, and is done by means of the stage micrometer alone, or with it in connection with the camera lucida. The stage micrometer, ruled to $\frac{1}{100}$ths and $\frac{1}{1000}$ths of an inch, is placed upon the stage of the microscope, the light adjusted, and the lines found first with the lower power and A eye-piece. An

ordinary flat ruler, marked to $\frac{1}{10}$ths of an inch, is then placed on the stage in front of the micrometer. The relation of the magnified and unmagnified spaces is then observed,—that is, how many of the unmagnified $\frac{1}{10}$ths are contained in the magnified $\frac{1}{100}$ths, whence the result is easily arrived at. Thus, suppose that $\frac{1}{100}$ in. on the micrometer equals $\frac{5}{10}$ in. on the ruler; then if $\frac{1}{100}$ in. magnified $= \frac{5}{10}$ unmagnified, a whole inch or $100 \times \frac{1}{100}$ will $= 100 \times \frac{5}{10}$, or 50. That is, the objective amplifies 50 times. The power of the same object-glass should then be computed with the B eye-piece, and then the magnifying power of the $\frac{1}{5}$th in the same manner; though for the latter the $\frac{1}{1000}$ths spaces of the micrometer should be selected, the liability to error with them being less when higher powers are computed. The process is even simpler when the camera lucida is used. The tube of the microscope is so inclined that the end of the eye-pieces is just 10 inches (the so-called nearest limit of distinct vision) from the horizontal table; the lines are then found and the cap of the eye-piece replaced by that carrying the prism, when the lines will be found projected upon the table. They may then be marked off, or the comparison at once made with the spaces on the ruler. In the use of the camera, the unemployed eye is best closed to prevent interference of surrounding objects, when, as the camera lucida is ordinarily constructed, one half of the pupil receives the rays from the object on the stage of the microscope bent to the eye by the camera, and the remaining half receives the rays from the table, revealing the table, paper, and pencil.

The arrangement for drawing is precisely the same, and after a little practice drawing with the camera becomes as simple as tracing.

THE MICROSCOPICAL STUDY OF BLOOD AND EPITHELIUM.

It is scarcely possible to study blood satisfactorily with a power of less than 300 to 500 diameters, such as is obtained with an American or English $\frac{1}{4}$ inch or $\frac{1}{5}$ inch object-glass with the B eye-piece, and it will often happen that a higher power will be of signal advantage. If a drop of the newt's or frog's blood be placed on a glass slide and gently covered with thin glass around the edges of which a little plain oil has been applied with a camel's-hair brush, a preparation is made which may be studied for hours.

The nucleated oval red blood disks of these animals will be readily detected; though it must be remembered that the nucleus is not at once visible in the blood just removed from the animal. In a few minutes, however, it makes its appearance, and grows slowly more and more distinct, until it becomes the characteristic feature of the red blood disk, and will always serve to detect it. In many disks, in a simple oil preparation the nucleus remains for a long time invisible.

More difficult of detection, as well as more interesting, are the *colorless blood corpuscles.* Of these, three and sometimes four forms are observable.

1. *The ordinary Amœboid Cell.*—A little careful examination, however, will soon enable the beginner to recognize one form of these corpuscles, here more easily than in human blood, because of its shape, at first distinctly round, or, more accurately speaking, *spheroidal,* as distinguished from the oval red blood disk. Its substance is transparent, faintly granular, and the corpuscle remains isolated, having no tendency to aggregate with others of the same kind, or with the much more numerous red disks.

This corpuscle being discovered, the attention should be fixed upon it, when it will soon be seen to become the seat of the well-known *amœboid* movements; as the result of which it changes its size and shape, becoming often three or four times as large, and sending out processes, some of which are short and prickly, others longer and attenuated, but terminating in a larger collection of bioplasm. In this manner also it effects a change of place, creeping slowly across the field of view. As soon as the corpuscle ceases to present a spheroidal form, there may be seen in its interior one or more roundish or ovoid bodies, which are the nuclei of the cell.

Some of these cells appear as though perforated in one or more spots by a "punch," an appearance which might on hasty observation be attributed to the presence of a nucleus, but it is more decidedly circular and sharply defined. These are called "vacuoles," and the white corpuscles containing them "*vacuole cells*." These "vacuoles" are changeable in size and distinctness, and are believed to be cavities filled with liquid due to the "constant commotion of the protoplasmic mass."

The blood of the cold-blooded animals affords a most convenient field of study for these cells, since no elevation of temperature is ever required to induce them. A large number of these cells can be demonstrated by placing a drop of newt's or frog's blood on a thin glass cover, which is laid, drop downwards, over a moist cell, made by applying a ring of putty of suitable diameter to a glass slide and placing a drop of water within its circumference, forming thus a moist chamber. As the process of coagulation proceeds, and the ring of serum extends itself beyond the periphery of the clot, numerous colorless corpuscles of the amœboid kind migrate from the coagulum into the serum, and may be studied in numbers in this situation.

One of Holman's *live slides*, so well known in Philadelphia, is admirable for the purpose. The inner concavity is filled with blood and the whole covered. In a short time the shallow outer circle will be filled with amœboid cells, which have wandered to the periphery.

The property possessed by amœboid cells, during the continuation of their locomotive movements, of incorporating in their structure particles of coloring-matter, milk globules, or even, according to Preyer,* particles of the red corpuscles of extravasated blood of amphibia, should be carefully looked for. The former may be studied by adding to a drop of blood a small quantity of liquid holding in suspension finely-divided particles of coloring-matter, as *vermilion* finely triturated in half per cent. solution of common salt, *carmine* dissolved in liquor ammoniæ filtered and precipitated by a small proportion of acetic acid, decanted and suspended in salt solution, or *fresh milk.*

2. *Granular Corpuscles.*—A second form of colorless corpuscle, the *granular*, also at first round, is promptly associated with its decidedly granular appearance, due to the presence of numerous large, distinct particles, whence the name. These also exhibit distinct amœboid movements more rapid than those of the ordinary amœboid corpuscle just described; but the granular cells are less numerous than the latter. In these, nuclei are sometimes visible even in the spheroidal form, but are more distinct after the cell has changed its shape.

3. In addition to these, smaller round cells, paler and less distinctly granular, are found in the blood of the frog and newt at all periods of the year. They correspond in size and character with the nuclei of the red blood disks.

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* *Pflüger's Archiv*, Jahrgang 1868, p. 423. Stricker's Histology, New Sydenham Society's ed., vol. i.

4. A fourth form of colorless corpuscle, which should be looked for in these bloods, especially in the spring and summer, is the *fusiform* or *linear-prolonged* cell, first described by Von Recklinghausen in Max Schultze's *Archiv*, Band ii. p. 137. They present a colorless cell-substance with a granular oval nucleus. The term "linear-prolonged" (Klein) is the more applicable, since they seldom, if ever, reach the elongated spindle shape we are apt to associate with the term fusiform, but are rather a "prolonged oval."

Although in these animals the amœboid movement is easily studied at ordinary temperatures, the application of heat greatly accelerates it, and makes it more decided. Increased temperature may be obtained by the careful application of the flame of the spirit-lamp to a metallic plate interposed between the stage-plate and the glass slide, or more satisfactorily by means of Stricker's warm stage. If the temperature, however, be raised above 40° C. (104° F.), the movements cease, and, according to Rollett, the cells harden.

EXAMINATION OF HUMAN BLOOD.

The method of examination of human blood is in no way different from that of the blood of the frog or newt. The circular biconcave shape of the red disks is well known. The "reversal of lights and shadows" due to this biconcavity, the centre of the blood disk acting as a biconcave lens, and the periphery as a double convex, should be thoroughly studied. Careful focussing will show that the centre is bright and the periphery dark when the object-glass is within the focus, and *vice versâ*, as should be the case on optical principles,—the centre dispersing and the periphery collecting the rays, so that in the former case the more remote negative

focus is to be focussed, and in the latter the nearer positive.

The *colorless* blood corpuscle is not so easy of detection in human blood as in that of animals whose red disks are round. The same general characters, however, belong to it. Its pale, faintly granular appearance, ordinarily round shape, indisposition to unite with others in the formation of rouleaux, and especially its *amœboid* movement, characterize it. The latter, even in human blood, in summer weather or moderately warm apartments, does not require heat to induce it; but it is accelerated by such addition.

Attention should be directed to smaller irregular collections of granular matter, apparently fragments, probably derived by fission or otherwise from the larger colorless blood corpuscles, and which are undoubtedly what have been mistaken by Salisbury and others for vegetable spores, and especially described by Zimmermann* as "elementary corpuscles," under an erroneous interpretation of their real nature.

In all colorless corpuscles the granules are subject to the well-known "molecular motion" or "Brunonian" movement, which may be studied with a power of 400 and upwards.

Reagents.—In none of the forms of the colorless blood corpuscle of the frog or mammalia is the nucleus, as a rule, readily detected without the addition of reagents,—at least while the spheroidal form is retained. Individual corpuscles are found in which this structure is differentiated; but it is by no means general.†

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* Rust's Magazine, vol. xlvi. p. 171.

† This is of course not due to any effect of the reagent in producing the nucleus, as has been stated by some, although the latter is altered by the former. That the nucleus is always present in the colorless corpuscle is proven by the use of high powers, in which, in consequence of the large

After a little practice with the blow-pipe and gas flame, one can make very delicate and convenient capillary tubes dilated at the middle, so that they approach a spindle shape. These are very convenient for receiving and dropping the various reagents in common use, as the indifferent salt solution, distilled water, dilute acetic acid, etc. They are really much more convenient than any other apparatus for dropping yet contrived.

1. *The Salt Solution.*—The first reagent with which blood should be studied is the "salt solution," a half per cent. solution of chloride of sodium, which is also called the "indifferent" solution, since it is intended to act indifferently as a substitute for blood serum, the natural menstruum of the blood corpuscle. *Serum* carefully drawn off from coagulated blood, or *aqueous humor* from the organ of vision, answers the same purpose more satisfactorily, the objection to the constant use of either being only in the difficulty of its preservation. The former may be kept in glass tubes for twenty-four hours, or in cool weather longer; but to avoid inaccurate observation the serum should be nearly fresh. The action of the serum and aqueous humor is truly indifferent,—that of the salt solution not altogether so. The former should produce no appreciable effect upon the red or colorless blood corpuscles; the amœboid movements should continue, and the other appearances remain. If human blood be diluted with salt solution, a marked change takes

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angle of aperture, a more delicate differentiation of light and shadow results, and in consequence the nuclei are always visible even without the use of reagents. This advantage of the high powers was pointed out to me several years ago by Dr Jos. G. Richardson. For the reason assigned, however, which may not be correct, I am alone responsible. I know of none better. The same is true of the nucleus of the oval red disk, of which we have said above that it is not generally visible by ordinary powers, while the disk is floating in the blood-vessels, or immediately after its removal from the body of the frog or newt. With high powers the nucleus, for the reasons above stated, is promptly discernible.

place in the red blood disk. It assumes the so-called crenated or horse-chestnut shape, in which radiated processes present themselves over the entire corpuscle, appearing as rays at the edges, but which when viewed from above appear as dots or dark points. On the colorless corpuscle the first effect of the impingement of the salt solution is to cause it to contract, to become smaller and more globular. From this shape, however, it immediately dilates as through elasticity, and the amœboid movements resume.

2. The next step is *irrigation with distilled water.* This is done by placing a drop of distilled water at one edge of the extemporaneous blood preparation, and at the opposite edge a narrow strip of bibulous paper, which is replaced by fresh strips as it becomes saturated. Thus is established a current from the drop of distilled water to the opposite edge, and the blood corpuscles are thoroughly "irrigated." If the colored corpuscles are to be studied, it is best to wait, as suggested by Klein, until they have shrunk; for we are then sure that many of them will have had time to sink, and adhere to the surface of the glass, and will therefore be less apt to be swept away by the current. The effect of this irrigation—the addition of a menstruum of less density than that in which the corpuscles normally float—is the absorption of such fluid by the latter. This imbibition must be attended by a change of form in the *red blood corpuscle*, which becomes spherical and smooth instead of biconcave. This change of shape is not always uniform, taking place much more rapidly in some corpuscles, and more rapidly in some directions of the same corpuscle, than in others. The spherical form is assumed because it is that shape which furnishes the greatest capacity. Watching the corpuscle thus distended, the color at first is present. Gradually, however, and some-

times rapidly, as the action of the water continues, the color disappears from the spheroids; they become faint, ill defined, almost invisible, and finally disappear altogether.*

In the nucleated oval corpuscles of the frog and newt, the nucleus comes at first more prominently into view, in contrast with the swollen paler periphery. Later, however, it also becomes swollen, smooth, and fainter.

Rollett, in Stricker's Histology,† calls attention to a structure which becomes developed in the elliptical corpuscles after the cautious addition of water. "The still ellipsoidal corpuscle is bounded by a perfectly smooth contour-line, but the place of the nucleus sometimes appears to be occupied by a colored spheroid, whilst in other cases numerous processes radiate from the ball towards the contour-line, becoming pointed peripherally." These forms, according to Kneuttinger, are obtained when fresh frog's blood from which the fibrin has not been removed is mingled with three or four times its volume of water, and an examination is shortly afterwards made of the resulting gelatinous mass.

In the instance of the *colorless corpuscle* the addition of water is also attended by some imbibition and swelling of the cell, the amœboid movements cease, and the corpuscle becomes spheroidal. The most marked effect is upon the granular matter and nuclei. According to Rollett,‡ the granular material contracts "around the nucleus," and only remains "connected with the surrounding parts by means of a few anastomosing processes." What-

* Here again, for the reason assigned in the previous note, the higher powers enable us to discern the framework of the corpuscle for a longer time after the coloring-matter has altogether disappeared,—affording another of the comparatively few instances in this department of microscopic inquiry in which high angle of aperture, rather than penetrating power, of which a low angle is a necessary condition, best serves us.

† New Sydenham Society's ed., vol. i. p. 396.

‡ Op. cit., vol. i. p. 58.

ever the precise changes may be, the granules finally disappear from the periphery and even close up to the nuclei, which become more distinct by contrast with the surrounding apparently empty space.

The nuclei themselves also at first swell, but finally, after considerable intermediate change of form, shrink up and become actually smaller. Finally the colorless corpuscle becomes disintegrated.

3. The action of *watery vapor* is similar, and is easily practised by affixing a putty ring to a glass slide, and placing a drop of water in the chamber thus formed. The drop to be examined is attached to the lower surface of the covering-glass, which is then brought down upon the ring of putty and carefully pressed in position. Some care must be taken in graduating the quantity of water, since, if too much be used, the under surface of the thin glass becomes covered with moisture; and if too little be used, the drop becomes dry before it is influenced by the vapor.

4. The action of *dense solutions*, as of neutral salines which do not precipitate the contents of the corpuscle, such as common salt, sulphate of soda, muriate of ammonia, borax, and others, as well as of simple syrup, should be observed. In consequence of their density, a current is established in the opposite direction from that induced by the water as a rarer fluid; hence the peculiar shape of the red disks is rendered more distinct; they become more biconcave, wrinkled, crenated, horse-chestnut shaped. In the colorless corpuscles, by the action of these solutions, the nuclei also slowly become more distinct.

5. *Dilute acetic acid*, say a twenty per cent. solution, is a reagent much used. Its action on the human blood-corpuscle is similar to that of water, but very much more rapid. Under its influence the

non-nucleated red disk swells up, becomes spherical, and finally loses its color, and, as described in the case of water, becomes almost and in some instances quite invisible; for a considerable time, however, before they disappear they present a more distinct contour than those acted upon by water.

On the colorless corpuscles, likewise, the action of acetic acid is similar to that described as due to the action of water, but more rapid. The difference in the effect of weaker and stronger solutions of acetic acid is one of rapidity only.

On the nucleated oval corpuscle of the frog and newt, acetic acid causes a beautiful staining of the nucleus with the coloring-matter of the blood, while a precipitate is apt to occur in the surrounding colorless substance.

6. *Alkalies* in sufficient concentration exert a solvent action upon both the red and the colorless corpuscles, causing their total disappearance, as is to be expected, since albuminous matter is soluble in alkalies.

In very dilute solutions their action, for the same reason, is not unlike that of acetic acid, swelling up the red corpuscles, making them spherical, and removing the coloring-matter, while in the colorless corpuscles the nuclei become more distinctly defined. In the action of the stronger solutions upon the nucleated oval corpuscles of amphibia, the nuclei are the last to disappear.

7. *Boracic acid*, two per cent. solution, has the same action as acetic on the human blood-corpuscle. On the nucleated oval red disks of the frog and newt it has a peculiar influence, first investigated by Brücke. If to a salt preparation of newt's blood this solution is added, the most conspicuous effect—described by Klein as a "coagulation of the hæmoglobin in the nucleus"—consists in an intense coloration of the latter apparently at the expense of the

coloring-matter of the remainder of the corpuscle, which becomes pale or completely colorless. This central body, which is at first oval, becomes larger, spheroidal, and yellow. It is called *zooid* by Brücke, and is sometimes beset with rays which radiate towards the circumference. The pale outside disk is also called by Brücke *oecoid* (οἶκος, a "house"), whence, according to him, the hæmoglobin previously inhabiting it is withdrawn and collects about the nucleus, forming the *zooid.* The same is said by Brücke to occur on the addition of a two per cent. solution applied to corpuscles dried on a slide, but has not been repeated by us. Moreover, this effect also occurs when, through the action of water, freezing, or electricity, the corpuscles have yielded up their coloring-matter to the serum, from which the coloring-matter seems again to be precipitated about the nucleus.

The boracic acid solution also, as in the case of water, and acetic and other dilute acids, causes the corpuscle to swell up, become ellipsoidal, and even spherical, the nucleus being often eccentrically situated; while the corpuscle even completely discharges its nucleus under the action of this acid.

8. *Tannin,* in two per cent. solution, produces a striking effect on red corpuscles, coagulating the hæmoglobin of the human disk into a knob-like mass (zooid). In the oval nucleated corpuscle of the frog a similar effect is produced, but the hæmoglobin is also coagulated about the nucleus, as with boracic acid.

9. Striking and beautiful effects are produced by the action of *carbonic acid,* as first demonstrated by Stricker, and may be studied without difficulty by accurately observing the following plan, derived from Dr. Klein in the Brown Institution, London. If Stricker's gas stage is not at hand, one may be extemporized in the follow-

ing manner. On an ordinary glass slide is placed a ring of putty; along the middle of the slide, extending from just within the putty wall, and perforating it, to a short distance beyond its edge, is attached a piece of fine glass tubing with a lumen of one-eighth to one-fifth of an inch. The tube is made to project beyond the edges of the slide, in order that a piece of rubber tubing of appropriate calibre may be slipped over it and attached to the gas-producing apparatus. At a point, however, between the edge of the slide and the gas-generator a T-shaped tube is introduced, the horizontal portion of which lies in the axis of communication between the wash-bottle and the slide, whilst the leg of the T is directed towards the observer (see Fig. 2 of the Introduction to Stricker's Histology, or Fig. 5, Pl. III., Sanderson's Hand-Book for the Physiological Laboratory). To this also is attached a long rubber tube, the end of which is placed between the teeth of the observer. The chamber, being constructed, is attached to the gas-generator of Stricker (see also same cuts). The latter consists of a flask of hydrochloric acid attached by a rubber tube to another containing small fragments of carbonate of lime, which is in turn connected with a wash-bottle, communicating by means of the rubber and glass tubing with the interior of the gas-chamber. It is evident that the generation of gas may be started or discontinued at pleasure by changing the level of the acid-bottle above or below that of the bottle carrying the carbonate of lime.

The apparatus being thus prepared, to—1. *a small quantity of human blood* on a glass slide is added *a relatively large quantity of the indifferent salt solution*. The two are mixed, and a drop of the mixture is placed on a circle of thin glass, which is then placed drop downwards over the putty ring, the

chamber placed on the stage, and watched until the corpuscles begin to appear crenated or "horse-chestnut shaped." The end of the tube between the teeth is closed, and a current of carbonic acid is passed into the cell by simply permitting the access of the acid to the carbonate of lime. *The crenated appearance promptly disappears.*

2. Take a *small* quantity of the blood of the frog or newt, and add a relatively *small* quantity of water, so that the disks become swollen but retain their color. Examine this blood, as before, in the gas-chamber. *No nuclei will be visible.* Allow carbonic acid to enter from the apparatus, and the *nuclei reappear.*

3. To a *small* quantity of the newt's or frog's blood add a relatively *larger* quantity of water than in (2), so that the corpuscles are much swollen but the color remains and the nucleus is visible. On the admission of carbonic acid the hæmoglobin *coagulates in the nucleus, coloring it decidedly.* If now by means of a clip on the gum tube the access of CO_2 be cut off, and the observer aspire through the tube in the mouth communicating with the T-shaped tube, the coloring (hæmoglobin) returns to the body of the disk, leaving the nucleus again uncolored.

4. To a small quantity of newt's blood add a *large* quantity of water, so as to remove all the color from the corpuscles. Then permit the access of CO_2, when a *precipitation of granules of paroglobin takes place* in the substance of the corpuscle. Now let the observer cut off the access of CO_2 by the clip, and aspire through the prolonged T-shaped tube to remove the CO_2, and *the homogeneous colorless appearance of the disk is again restored.*

TO MAKE HÆMIN-CRYSTALS.

The preparation of *hæmin-crystals* is a much simpler matter than is commonly supposed. A drop of human blood is placed upon a slide and allowed to dry. A little of this is scraped off, placed upon a second slide, a few particles of common salt added, and covered. Two or three drops of glacial acetic acid are then allowed to pass under the thin glass cover. The slide is heated for a few seconds over a spirit-lamp (until a few air-bubbles appear is sufficient), and examined by the microscope. Hæmin-crystals will be found.

EPITHELIUM.

Ciliated epithelium may be obtained for examination by scraping the back part of the tongue or throat of the frog or toad, and should be examined in the salt solution. Thus mounted, the ciliary movement may be studied for hours.

Ciliary motion having ceased, it may be temporarily restored by the addition of any reagent, as water, solution of potash, acetic acid, carbonic acid, etc. This temporary restoration of the movement is, however, simply the result of currents induced by the liquids thus added, as will be plain a few minutes later, when, coming into *actual contact* with the cells, they *promptly cause cessation of the ciliary movement.*

In a preparation thus made, some cells will be seen to be very much more granular than others. Any cylindrical epithelial cell from mucous surfaces may become thus granular by the addition of water, chromic acid, and other reagents; pressure, and, therefore, accident, may induce them. Certain of these cylindrical epithelial cells thus acted upon by

water should be further carefully watched; eventually hyaline spherical knobs appear, apparently coming from the anterior edge of the cell. Finally liberated, they leave the cell whence they are derived, with a concavity, whence it is termed a "goblet cell." These goblet cells are considered by some as peculiar mucus-forming cells; but, for the reason just given,—that any epithelial cell from these sources is liable to take this shape,—they are justly denied by Klein to possess any such special function.

If it is desired to preserve epithelium for study, portions of the fresh tissue may be taken from different localities, as the trachea, tongue, intestine, bladder, etc., and washed in a very dilute solution of chromic acid, say one-eighth to one-fourth per cent. They may then be kept for a long time in a solution of one-eighth per cent. strength, for a longer time in a strength of one-tenth per cent., and, if it be desired to keep them for a short time only, a strength of one-half per cent. may be used. Very suitable for hardening and preserving these delicate cell-structures is "Müller's eye-fluid," composed of bichromate of potash 2–2½ grammes, sulphate of soda 1 gramme, distilled water 100 grammes. The tissue should be exposed to its action at least two weeks.

1. Columnar epithelial cells including ciliated cells may be obtained from the trachea of the rabbit.

2. Pavement-epithelium from the tongue of the rabbit, and among these "ragged cells," or "riff cells" (Riefezelle, Stachelzelle).

3. Pavement and club-shaped cells (intermediate forms) from the urinary bladder of the rabbit. The excavated under surface of the former, into which the latter fit by their upper convex extremities, should be noted.

4. Columnar epithelium from the stomach of the rabbit.

5. Columnar epithelium on the villi of the small intestine of the rabbit; the pale striated rim on the free border should be looked for. These striæ, which can only be satisfactorily studied with the higher powers particularly of the immersion system, are generally acknowledged by histologists to be due to delicate porous canals which perforate the seam.

They are best shown in *fresh* preparations from the intestine of cats and dogs, mounted in serum.

6. The epithelium from the intestine of the fresh rabbit should also be examined after feeding the animal, with a view of detecting oil-globules in transit through it.

CONNECTIVE TISSUE.

For this purpose a small portion of the mesentery of a living frog is placed upon a slide in a drop of serum or one-half per cent. solution of chloride of sodium, and covered with a thin glass cover. Five forms of cells are met with in connective tissue.

1. The *wandering cell*, morphologically identical with the white blood corpuscle, and exhibiting its peculiar amœboid movement resulting in a change of shape, to which is added, also, a change of position. This connective-tissue corpuscle assumes a permanently round form on the addition of water, when it may be said to be dead. These, in some instances at least, are probably white blood corpuscles which have migrated through the walls of blood-vessels into the connective tissue.

2. The well-known, coarsely granulated (grob-granulirt) *spindle-shaped* connective-tissue corpuscle, provided with an elliptical nucleus.

3. The *round, finely granulated* (fein-granulirt) connective-tissue cell, which exhibits no movement, or at least a very slow one, and differs only from the dead amœboid cell in its larger size. These generally have an oval nucleus, or, according to Rollett, their contents may appear to be accumulated at one point around a body resembling a nucleus; according to this observer, also, they give off radiated prolongations which often join. Between the spindle cell and the round cell are intermediate shapes.

The same study may be made in the connective tissue derived from the fresh muscle of the thigh or leg of the frog or rabbit; but the cells are less numerous.

4. In the connective tissue of the muscles of mammals, *cylindrical* (walzenförmig) cells are sometimes found, containing an elliptical nucleus.

5. Finally, *pigment-cells* are also found in connective tissue, abundantly in amphibia and fishes, but to a limited degree only in man (iris, choroid, and innermost layers of the sclerotic). These are usually provided with a central nucleus, often obscured, and also exhibit movements, which are, however, exceedingly slow in adult batrachians, but more rapid in their embryos.

Rollett, in Stricker's Histology (New Syd. Soc. translation, p. 57), describes other finely granular cells "which are for the most part more delicate and pale, and frequently exhibit fine radiating strongly refracted striæ of greenish tint. These easily overlooked, delicate, and proportionately large structures may be best distinguished by their very distinct, large, vesicular nuclei." I have had no opportunity of recognizing these cells as special forms.

The addition of very dilute acetic acid, so dilute as to be just appreciably sour to the taste, causes

an increased granular appearance in these cells. A stronger solution, say .20, causes a more distinct exhibition of the nuclei, which in the case of the migratory cells are round and smaller, and frequently exceed one in number, while the nuclei of the other connective-tissue cells are *oval* in form and larger in size. The addition of still stronger acetic acid produces shrivelling in the cells, a formation of folds, and extrusion of the nucleus in the case of the wandering cells.

One or more of these cells are met in *two principal varieties* of connective tissue: 1. Trabecular tissue (Balkennetz), adenoid tissue of His, embryonal tissue. 2. Fibrillar connective tissue.

1. The *trabecular connective* tissues are generally characterized by the fact that they do not yield gelatine on boiling. They form net-works of which the trabeculæ were primarily produced by the union of radiating processes of cells. These may preserve their cellular character in retaining the nuclei of the cells, producing thus a nuclear trabecular tissue, or the trabeculæ may so acquire a fibrous constitution as to be undistinguishable from bundles of the fibrillar variety of connective tissue. This variety of connective tissue may occur in independent masses, as in the so-called mucous tissue of the umbilical cord, or it may serve as a means of support, or investment of protection, for fibrillar connective tissue, or vascular (capillary) tissue, or organs, as in the lymph-glands and similar structures, Peyerian and solitary glands, the mucous membrane of the intestines, tonsils and follicles of the tongue, the conjunctiva and its trachoma glands, and the nasal portion of the pharynx. In these latter instances the meshes are filled with the peculiar lymphoid (amœboid) cells described. The connective tissue is also traversed by blood-vessels, and unites upon the external surface of the blood-

vessels to form the tunica adventitia capillaris of His.

It is this tissue which constitutes the neuroglia of Virchow, the delicate supporting connective tissue of the eye and nervous centres. It also occurs as a constituent of certain pathological new formations. This form of connective tissue is best demonstrated, according to the method of His, by carefully brushing thin sections of the hardened tissue with a camel's-hair brush, by means of which the lymphoid cells are removed, and a delicate net-work is left behind, composed of trabeculæ enclosing round or polygonal spaces: these trabeculæ are sometimes distinctly seen to be composed of nucleated cells or to exhibit nuclei, or, in consequence of age, to have lost all cellular characters.

2. *Fibrillar Connective Tissue.*—Between the trabecular connective tissue or "Balkennetz" and the fibrillar form are intermediate forms; but the great bulk of the connective tissue of the higher animals at least, is made up of the fibrillar variety.

This—to which alone the term connective tissue was formerly applied—is transformed into gelatine on boiling, and is characterized by the well-known fibrous structure implied in the term fibrillar. The cellular elements are those already described, and can only be satisfactorily studied in the young formation of this tissue when quite fresh.

The toughness or close adhesion of many forms of this connective tissue, as in the cornea, sclerotica, tendons, fascia, and even subcutaneous tissue, is well known. It is often so great as to make it impossible to isolate the fibrillæ composing it, without the use of reagents. One of the best of these is *simple lime-water.* A piece of sclerotica or subcutaneous tissue, for example, is thrown into lime-water and allowed to remain for twenty-four hours. At the end of this time a small piece is taken out

and frayed with needles. If it stubbornly resists the dissection, it is replaced in the lime-water for another twenty-four hours, when the dissection is repeated. Six or seven days of maceration are often required, when the dissection can be made with surprising facility, and beautiful preparations may be obtained in which the separate fibrillæ may be clearly displayed. The sclerotic and cornea of the rabbit and the subcutaneous connective tissue from the dog—which is very tough and difficult to fray out—are thus successfully manipulated.

A similar agent is *baryta-water*, acting more promptly than lime-water, and accomplishing the same results in from four to six hours.

A *ten per cent. solution of chloride of sodium* is also very suitable. A maceration of fifteen minutes for a small tendon suffices to render the fibrillæ easily isolable.

A fourth reagent, which acts similarly to the lime-water, though not so rapidly,—indeed, very slowly,—is a two per cent. solution of *bichromate of potash*. The maceration may be continued two or three weeks, according to the firmness of the tissue, and the solution should be often renewed. The fibrillæ are thus rendered easily isolable. The use of this solution is attended by a yellow coloration of the fibrillæ, which may or may not be desirable. This solution is used successfully in the treatment of the adventitia of blood-vessels and in the case of tendons.

The rationale of these processes is not precisely known; though it is usually said that the lime-water and bichromate of potash dissolve a species of cement (Kittsubstanz) by which the fibrillæ are believed to be held together.

Very interesting are the effects of acetic acid and boiling water upon the fibrillar connective tissue. Acetic acid of almost any strength beyond .01, if

sufficient time is allowed, removes completely the fibrillar appearance of this form of tissue, rendering it homogeneous and transparent, and increasing the width of the bundles, while the cellular element and the cord-like fibres of yellow elastic tissue are rendered more distinctly visible. On the other hand, the addition of a little weak solution of ammonia promptly restores the structural characters. The most convenient solution for general use is a .20 solution of the pure glacial acid. This may be further diluted, if required.

Again, a piece of subcutaneous connective tissue should be boiled in water in a test-tube for five or ten minutes. There results the same absence of fibrillar appearance, with increased width of the bundles, and constrictions at certain points where the spirally *surrounding* fibrillæ still remain, not permitting the swelling to take place in these situations. Longer boiling dissolves or causes the disappearance of these surrounding fibres also, and if continued still longer causes a solution of the entire mass into gelatine. Tendon may be also similarly treated, and with the same results. For these purposes the hot-water stage of Stricker may be used to great advantage.

The staining of connective tissue is attained by three principal staining solutions, *carmine*, *chloride of gold*, and *hæmatoxylin*.

Carmine staining is accomplished by making an ammoniacal solution of carmine, without particular regard to strength, and diluting by water or glycerin to such degree that if a small quantity is placed in a watch-glass a printed page beneath it will still be legible. The tissue is put into a small quantity of this, and examined from time to time, to determine the degree of staining. When the proper time of immersion for a given fragment is determined, others of the same kind and size

are immersed for a corresponding period. In all instances an intensity or vividness is secured by further treating the stained tissue by a very weak solution of acetic acid, of a strength just sufficient to allow the acid to be tasted, say five drops to the ounce, or even less. This staining is not restricted to any one element of the tissue, but is general, the cells being more deeply tinted than the fibrillæ, and of the cells the nucleoli, if present, are most deeply tinted, the nuclei less, and the cell-contents least. If the staining should happen to be too deep, the excess may be dissolved out by a weak solution of hydrochloric acid. With care the carmine may thus be entirely removed from the fibrillæ, if desirable, leaving only the cells stained.

Staining connective tissue with *chloride of gold* has for its object more particularly the demonstration of the cells of the connective tissue, and is practised for this purpose in the case of delicate connective tissue. A piece of the mesentery of a frog is carefully cut out and stretched by means of pins over a fenestra cut in cork. Thus prepared, it is placed in a one-half per cent. solution of chloride of gold for ten minutes, after which it is removed to distilled water slightly acidulated by acetic acid, where it is allowed to remain twenty-four hours, in order that the gold may be reduced and the cells stained. The preparation when taken from the acid should be of a uniformly violet color; it will also be somewhat hardened. It is then examined in glycerin. The different forms of connective-tissue corpuscles should be noted, and care should be taken not to mistake for them the nuclei of clumps of the red corpuscles of the frog, which are apt to be encountered in consequence of section of a blood-vessel.

The period of immersion cannot be positively stated: experiment must determine the exact time.

Ten minutes is perhaps an average: larger preparations require longer, the more delicate a shorter time.

So far, however, as my own experience goes, I am not disposed to assign a very high position to the gold staining of connective tissue. The cells only are stained, and that somewhat faintly, and the preparations cannot be mounted to keep any length of time; while the vividness and permanence of carmine stainings, when mounted in glycerin or slightly acid glycerin, are well known.

Hæmatoxylin staining is described on page 41.

Making Sections of Tendon and other Tissues.—The imbedding process is usually employed; the substance made use of for the purpose being white wax or paraffine reduced in consistency by oil, the two being melted together. The consistency to be obtained is one approaching that of the tissue to be cut after hardening, while the quantities employed to attain it must be influenced by temperature, the season of the year, etc. Of oil and wax the average test proportion is half and half, of paraffine and lard 5 to 1. The preparation is always previously hardened in alcohol or chromic acid, and in the case of tendon it is sometimes dried, when indeed it may be cut without imbedding, though the change undergone by a specimen thus treated is manifestly great. The process employed is very simple. A little box is made of moderately stiff white paper, and adapted in size to the preparation to be cut. The box is made in the following simple manner. A rectangular piece of paper is folded as indicated by the dotted lines and cut as shown by the plain lines in the diagram; this permits the corners to be turned up and about each other, so that, being pasted in this position, a neat box is formed. The preparation is then taken from the fluid in which it was immersed (the excess being allowed to evaporate, or removed by

bibulous paper), and perforated towards one end by a pin, which is then thrust into the floor of the box in such a way that the preparation occupies a central position, so that the molten matter may readily flow around it. The mixture of wax and oil is then melted over a spirit-lamp, and allowed to cool until a slight film begins to appear at the edges. It is

then poured rapidly around the preparation and allowed to cool. If it is desired that the cooling take place rapidly, it may be placed in water; but, if possible, it should be allowed to cool gradually. After the mass is cool, the paper is stripped off. We then have a rectangular prism, of convenient size to hold between the fingers. This is cut with an ordinary knife until the preparation is reached, when sections are made with a broad, flat knife mounted like a razor, which is much used in Vienna, the imbedded tissue wrapped in a towel being held in one hand and the knife in the other. No precise directions can be given as to the mode of making the sections: experience alone can teach this. It must not be omitted, however, that the knife and upper surface of the preparation should be wetted in each case with alcohol or water, according to the previous immersion. So also the section is

floated off the knife in alcohol or water, according to its previous treatment. It may likewise be stated that it is not necessary to cut entirely through the imbedding substance, but that it is better to start the knife just beyond the edge of the specimen to be cut.

After the sections are made, they may be stained in chloride of gold or carmine or other staining fluid, in the manner described. Or if the tissue is a delicate one, as certain almost thread-like tendons of the frog, it may be stained before the sections are made.

A paper box for imbedding may be more quickly made by means of a metallic cylinder (say of brass) an inch in diameter and half an inch or an inch long, about which a piece of stiff paper of the required length of the box may be rolled, one end turned under the cylinder, and the overlapping edges pasted in position. The brass cylinder may be removed, or allowed to form the bottom of the box in which the specimen is fastened, imbedded, and cut as before.

A medium very suitable for imbedding, which I have used at the suggestion of Dr. J. G. Hunt, of this city, is the ordinary transparent soap of the shops, which is cut into small pieces and melted in a water-bath with the aid of alcohol. It may be prepared in considerable quantities, poured into a bottle, and when desired for use the bottle need only be placed in a vessel of water for a few minutes, when the imbedding material is ready for use. Its advantages are its cleanliness and transparency.

Nothing is said of the numerous forms of section-cutters provided for this purpose, for, although very convenient, they are not necessary.

Unstriped muscular fibre is studied in the following manner. A section of fresh intestine of the dog or of the rabbit is taken, washed by shaking about gently in a very weak solution of bichromate of potash, and then placed in a dark straw-yellow solution of bichromate of potash (about .02) for three days, during which the solution should be changed at least once. This loosens the connective-tissue net-work, as well as the net-work of muscular tissue, *i.e.*, renders the fibres easily isolable. (Any weak acid, as a .20 solution of acetic, hydrochloric, or nitric, answers the purpose, but the bichromate of potash solution is better.) The muscular coat is then stripped off quite easily, and small portions dissected in the fluid in which the tissue has been macerating. Very beautiful nucleated muscular fibre-cells may thus be obtained, which possess a yellow tinge in consequence of staining by the bichromate of potash.

Of *reagents* a one-half per cent. solution of chloride of gold followed by a .01 solution of acetic acid renders the cells and their bundles more distinct, and the contours of the fibre-cells are more easily followed. Dilute acetic acid renders the nuclei more distinct.

To stain unstriped muscle.—Very beautiful preparations are made by staining, especially with chloride of gold, muscular membrane—as a portion of the stomach or small intestine of the rabbit—and making sections of the same, which should be made both parallel and transverse to the length of the muscular fibre. In these and all other instances in which preparations to be stained with chloride of gold require washing, this should be done by shaking it about

gently in a much weaker solution of the gold, or, what is cheaper, a very weak solution of tartaric acid. The mucus-membrane must be stripped off with forceps, and the gold staining is then accomplished as described, by mounting the membrane over a fenestra in cork, previous to immersion in the one-half per cent. solution, transferring to acetic acid water, and further hardening in alcohol if the preparation is not sufficiently hard when removed from the acidulated water. The sections are made as before described. It will be easily seen that very different pictures will be obtained in the transverse sections according as they are made through or beyond the nucleus. Beautiful net-works of nerves traversing the muscular lamellæ will also be stained by the chloride of gold. In all cases where sections are not sufficiently transparent, they may be made so by immersing for a few minutes in turpentine, or better in oil of cloves, to either of which they may be transferred from alcohol.

Striated muscular fibre may be studied in several ways. 1. By maceration for forty-eight hours, more or less, according to the size of the fragments, in the two per cent. solution of bichromate of potash. It is then dissected with needles and examined. The fasciculi are rendered easily isolable, but are colored somewhat by the bichromate solution, and altogether more altered than by the following methods. 2. Warm small pieces of muscle in a capsule or test-tube, containing equal parts of water and one-half per cent. solutions of nitric acid and chlorate of potash, for seven minutes, without boiling. As soon as the fluid begins to vaporize, remove the lamp to a greater distance, but keep up the heat for seven minutes; then transfer to distilled water. The fasciculi become most easily isolable, and may even be shaken apart. The connective tissue is thus dissolved, but less completely

than in the next method, and the nuclei of the sarcolemma, as well as of the muscle substance, are distinctly seen. The latter should be compared with the former. The nuclei in the sarcolemma of muscle are usually smaller than those of the muscular substance, and the latter oftener contain two nucleoli. 3. Boil a fragment of muscle for five minutes in a test-tube, in one part of one-and-a-half per cent. solution of nitric acid, one part of one-and-a-half per cent. solution of chlorate of potash, and ninety-eight parts of water. Place the muscle thus boiled in distilled water twenty-four hours, changing the water once. In this method the connective tissue is completely removed, and even the nuclei of the sarcolemma become invisible.

The distribution of nerves in striated muscle should be studied. This, in animals, is best done in the fresh muscle of those killed by woorara. Much patience is required, and many preparations must be examined in the so-called indifferent solution—one-half per cent. chloride of sodium—before nerves may be detected and any information with regard to their terminations can be obtained. In the fresh muscle of coleoptera, however, as that of the water-beetle, *hydrophilus piceus*, the termination of nerves in the so called Doyerian plates (*hügel*), and the continuation of the sheath of Schwann (tubular membrane) with the sarcolemma of the muscle, are easily demonstrated. The contraction of muscle is also here beautifully shown.

The *sarcolemma* of muscle is best demonstrated by adding water to a fresh preparation of frog's muscle, whence the sarcolemma soon separates in transparent double-contoured bulgings. By careful teasing with needles, the sarcous substance may be ruptured, and the sarcolemma remains intact.

ALIMENTARY CANAL.

Very beautiful sections of the alimentary canal of the dog—œsophagus, stomach, and small intestine —may be made by first washing the portion in the very weak chromic acid solution alluded to, hardening in the half per cent. solution of chromic acid for forty-eight hours, transferring to alcohol for two days, and then making sections in the manner described. These may or may not be afterwards stained with carmine. The yellow coloration by the chromic acid serves also to give the different coats a distinctness not obtained with uncolored preparations. In the case of the small intestine the following layers can be demonstrated with perfect facility. 1. *Epithelium.* 2. *Membrana mucosa.* 3. *Muscularis mucosæ*, or internal muscular coat, composed of two layers,—*a.* circular, *b.* longitudinal. 4. *Submucous connective tissue.* 5. *Muscularis externa*, also composed of two subdivisions,—*a.* circular, *b.* longitudinal; and finally, 6. *The peritoneum.*

STAINING OF TISSUES.

No auxiliary has so greatly aided the histologist as the staining process. The method of *carmine staining* has been discussed under connective tissue.

Gold Staining. — The general method of gold staining has also been given. Certain modifications must of course be made for special tissues and special objects, as demanded by their size or shape or the points to be demonstrated. Thus, in staining corneæ the period of immersion is longer if it be desired to stain the nerve-fibres than if simply to study the so-called corneal corpuscles, and corneæ of rabbits require, of course, longer immersion than

those of frogs. For the former an immersion of as much as three-quarters of an hour is sometimes necessary for staining the corpuscles, and one and a quarter hour for nerves. For the latter an immersion of from ten minutes to half an hour may be allowed before transferring to the acetic acid solution; the former for corpuscles, and the latter for nerves. In either case the epithelial membrane of Descemet may or may not be removed by pencilling with a camel's-hair brush, or by careful traction with forceps.

In the case of the rabbit's cornea especially, the stained corneæ may be imbedded and sections made.

Washing is often necessary in order to remove coagula formed by irregular precipitation of the staining substance. This is done by shaking the stained tissue in a test-tube with alcohol, water, turpentine, or other menstruum with which it was last treated.

Again, if it is desired to stain the epithelium and submucous tissue of the intestine of a small animal, as a rabbit, without section of the gut, a portion of the intestine may be filled with the one-half per cent. gold solution, by means of a rubber tube with attached pipette, and allowed to remain thus for ten minutes; then empty and place in the acetic acid solution for twenty-four hours. Further harden, if necessary, in alcohol, imbed, and make sections either of the entire cylinder, or of smaller portions of it, as may be desired.

Beautiful *nerve-preparations* may be made of the tadpole's tail, cutting off a portion, washing in the very weak chloride of gold solution or solution of tartaric acid, staining with the one-half per cent. solution, imbedding, and making sections.

One of the most beautiful manipulations for demonstrating *nerves* and *unstriped muscular tissue* is to

fasten a frog on its back, open the abdomen, first inflate the bladder through the common opening of the bladder and rectum, then fill the bladder with the gold solution by means of the rubber tube and attached pipette, allowing it to remain for five minutes, while the exterior of the bladder is kept exposed to a similar solution for the same length of time. Portions may then be cut out and examined in glycerin.

The distribution of nerves in the mucous membrane of the vagina, now claiming considerable attention, is also thus studied. Small portions of mucous membrane are cut out of the vagina of the rabbit, suspended over a fenestra in cork, stained with chloride of gold solution, transferred to acetic acid water for twenty-four hours, and studied in glycerin.

Unfortunately, gold-stained preparations cannot be preserved for any length of time. They are best studied in glycerin, but mounted in this fluid they cannot be relied on for more than a month; though if the cover be cemented by damar lac, they keep even longer. Mounted in oil of cloves, to which they may be transferred from alcohol, and cemented by damar lac, they keep somewhat longer, even a year or more, with little change.

Silver Staining.—It is well known that "silver staining," so called, has this peculiar action, that it affects, not the cell-substance, but a certain intercellular cement, by which the cells seem united, and that it maps out, therefore, districts of cells by staining their periphery. It has its greatest utility in demonstrating the endothelium of blood-vessels, and especially of lymphatics, in serous membranes (centrum tendineum of the diaphragm, pericardium, etc.), but is also used in cornea-staining, in which it marks out the tortuous periphery of the peculiar corneal corpuscles. It is accomplished either by pen-

cilling the organ or tissue of the living animal with a one-half or one-fourth per cent. solution of nitrate of silver, repeating once, keeping in the dark for ten minutes, and then placing in distilled water for twenty-four hours, renewing the water once; or, secondly, by removing the organ either from the living or recently killed animal, immersing in the silver solution in the dark for from one to ten minutes, and transferring to distilled water for twenty-four hours. In all instances the epithelium must be previously removed from the tissue or organ to be stained; which is very easily done by the simple friction of a camel's-hair brush, or by wiping the epithelial surface with a dry cloth. The most successful preparations of this kind I have ever seen were made from the central tendineum of the recently killed rabbit, by Dr. Klein, at the Brown Institution, London. The demonstration of the lymphatics and lymph-spaces was perfect. The preparations are best examined in glycerin, in which they may be preserved for a short time. Nitrate of silver injections of small blood-vessels, as those of the kidney of a rabbit, are also made by means of delicate glass pipettes connected by an India-rubber tube with the syringe carrying the one-half per cent. solution. The manipulation is delicate.

Hæmatoxylin Staining.—Perhaps the most satisfactory process of staining for general demonstration, especially where the tissue has been hardened in Müller's fluid or chromic acid, is that by hæmatoxylin, introduced by C. F. Müller in his researches upon the cornea, and into the country by Dr. J. W. S. Arnold, of New York.* When carefully practised, the differentiation of structure due to different degrees of coloration is more successfully attained than by the other methods, the nuclei and younger

* *Philadelphia Med. Times*, July 1, 1872.

bioplasm being more deeply tinted than the cell-wall, intercellular substance, etc., of earlier formation. The tint obtained is similar to that produced by chloride of gold.

It may be practised as follows: a solution of hæmatoxylin and alum is made, the latter substance being required as a mordant, say according to the following formula:

Ext. hæmatoxylin, ʒiv;
Alum and potas. sulph., ʒij;
Water, fʒij.

Mix and filter, when a deep violet solution is obtained. This is far too strong for ordinary use, and may be diluted with ten or twelve times its bulk of water or glycerin. Alcohol precipitates from this solution, and therefore is not suitable; and glycerin is better than water even, though with it the staining takes place more slowly. It is better that the solution be too weak than too strong, as a longer exposure will accomplish the same objects as a stronger solution, and if the solution be too dark an opacity results which will ruin the specimen; though an excess of hæmatoxylin may be removed by the cautious use of dilute chlorine water. The length of time required depends on the character of the specimen. It is advised, as in the case of carmine, to test with sections, and thus determine the time required for each set.

Fresh tissues require more time than those which have been hardened in alcohol or chromic acid. They should be washed in water or alcohol, and may be mounted in glycerin, damar lac, or oil of cloves.

There are other agents used for staining. Anilin is valuable for its rapidly staining properties; but it is not permanent. A suitable red may be obtained by the following formula from Frey:

Anilin red (fuchsin) crystallized, 1 centigramme;
Strong alcohol, 20 to 30 drops;
Distilled water, 15 cubic centimetres.

A beautiful blue is obtained by first treating the insoluble anilin blue with sulphuric acid, and dissolving 1 centigramme of the resulting soluble product in 25 cubic centimetres of distilled water and 20–25 drops of alcohol.—*Frey and Klein.*

NERVOUS TISSUE.

The two elements of nervous tissue, the *fibrous* and the *vesicular*, require separate consideration.

1. *Nerve-fibres.*—If a nerve be examined in its unaltered state, it is scarcely possible to make out the three elements, *axis-cylinder*, *medullary sheath*, and *Schwann's sheath*, of which each nerve-tubule in a nerve-trunk is composed. On removal from the body, however, and teasing with the needles on a glass slide, changes are very rapidly undergone which facilitate the demonstration. If it be desired to study a nerve as it appears in the living body, a small portion of the sciatic nerve of a frog, or, if accessible, a piece of the radial nerve from a human subject recently dead, is removed and rapidly teased in salt solution or iodized serum.* Thus examined, besides a very considerable amount of connective tissue forming the neurilemma or sheath surrounding the nerve and binding the bundles of tubules together, a number of smooth highly refractile bands will be seen, which exhibit, owing to this

* *Iodized serum*, highly recommended by Max Schultze for the study of fresh tissues, consists of the amniotic fluid of the embryo of the ruminantia, to which about six drops of a concentrated solution of tincture of iodine is added while shaking the mixture. A "wine-yellow" colored solution results, which becomes paler, and may even require a few drops more of the iodine tincture after a time. As a substitute, an artificial mixture composed of 1 ounce of egg albumen, 9 ounces of water, and 2 scruples of chloride of sodium, with the proper proportion of tincture of iodine, is suggested by Frey.

refraction, a dark border and bright centre. These are the nerve-tubules, of which, however, nothing is really seen but the *medullary sheath*, or white substance of Schwann, immediately embracing the axis-cylinder, obscuring the latter as well as the Schwann's sheath, a tenuous transparent covering outside of the medulla. So characteristic is this appearance that its presence is always considered indicative of the presence of nerves. Almost immediately, however, if water is used as the menstruum, in a longer time in salt solution, and not until several hours in iodized serum, the nerve-tubule begins to lose its smoothness and to become beset with drop-like bodies of irregular size and form, some of which are transparent and some granular. This is said to be due to a coagulation of the medulla. But this very coagulation and consequent distortion permit a view of the essential part of the nerve-tubule, the *axis-cylinder*. This is best seen at the ends of the tubules whence the medulla has retracted in its coagulation and left the axis-cylinder bare. Glimpses of it may also be seen between the drops formed by the coagulation of the medulla.

But the axis-cylinder is more satisfactorily studied by taking portions from the lateral columns of the spinal cord of a cat or other small animal, the optic, olfactory, or sympathetic nerve, which has undergone maceration for several days in a one per cent. solution of bichromate of potash. Teased out in some of the same fluid, axis-cylinders are found in abundance whence the medulla has been retracted.

The *fibrillar structure* of the axis-cylinder should be sought for in these preparations. Klein gives the following method of demonstrating the fibrillar structure of the axis-cylinder in any nerve. A piece of fresh nerve is put in common alcohol for a few minutes, and then stained with carmine. (The axis-

cylinder is alone readily stained.) It must then be put into absolute alcohol for twenty to thirty minutes, after previously teasing it out somewhat. It is allowed to remain twelve hours or more in oil of turpentine, and then covered in damar varnish, when it will be found that all the nerve-fibres are more or less completely deprived of their medullary sheaths. On examination, the axis-cylinder appears in general to consist of granulous substance, but here and there distinct longitudinal streakings can be recognized.

The *Schwann's sheath*, or external tubular membrane, is not easy of demonstration, and generally only the nuclei can be seen; and these are liable to be confounded with the nuclei of the neurilemma. We are informed by Klein that "in the nerves of the tail of the tadpole, and of the membrana nictitans of the frog, in those of the mesentery of the frog and of mammalia, whether in the fresh state or treated with gold, in the corneæ of the frog or of mammalia treated with gold, in sections of the epiglottis or of the mucus-membrane of the mouth after treating the tissue with gold, the Schwann's sheath can often be recognized as a more or less distinctly streaked membrane."

The demonstration of the *non-medullated* nerve-fibres by the chloride of gold staining has already been described. Hæmatoxylin also beautifully displays them in thin membranous tissues, as the mesentery or bladder of the frog.

2. *Nerve-cells* may be studied in the ganglia on the posterior roots of the spinal nerves and in some cranial nerves; also in the gray matter of the brain, in the central gray matter of the spinal cord, and in the ganglia of the sympathetic. These ganglia may be hardened in one-half per cent. solution of chromic acid or Müller's fluid, may be cut and stained as described, or, better, teased out with needles. They will

be found to consist of a capsule and trabecular network of fibrillated connective tissue, with its cellular elements supporting an abundant vascular system and the so-called *ganglion cells*. These are found to be granular, sometimes fibrillated, distinctly nucleated and nucleolated, and provided with the characteristic polar prolongations. In the spinal nerve-ganglia of the mammalia only unipolar cells, usually of small size, are found. The ganglion cells of fish and frogs are usually bipolar, but also multipolar. The ganglion cells of the anterior horns of the spinal cord of the calf are very large, and have a granular substance in which with high powers fibrillæ may be distinguished. They have large round double-contoured nuclei distinctly nucleolated, and possess processes of two kinds, the so-called "axis-cylinder process," and the branched "protoplasma processes" of Deiters, by whom they were first investigated. "The axis-cylinder process springs from a broad base, from which it tapers to a fine filament. To whatever distance this filament is traced, it is seen that it does not branch, but becomes thicker and eventually assumes the character of a medullated nerve-fibre. The other processes are broad and flattened, and soon divide dendritically. They consist of fibres imbedded in a coarsely granular interstitial substance; the fibrils can be distinctly followed into the ganglion cell." (Klein, English ed., p. 7.) These processes, forming a dense net-work, are probably in direct continuity with the nerve-fibres which enter the cord by the posterior roots. The cells of the posterior horns are similar, but smaller. For admirable drawings of these cells, see Stricker's Histology, New. Syd. Soc. transl., vol. i., Frey on the Microscope, and Plates by Klein to Burdon-Sanderson's Hand-Book for the Physiological Laboratory.

BONE AND TEETH.

There are two conditions in which bone and teeth are made the subjects of microscopic study: 1, in thin sections of partially decalcified tissue, still retaining, however, all of the morphological elements of the living structure; and 2, in thin sections of dry dead bone and tooth, which are merely the framework of the living structure, though affording very beautiful objects for microscopical examination. It is needless to say it is the former that should become the object of investigation by the student; and, fortunately, it offers us the shape most easy of manipulation.

To prepare sections of living bone and teeth, portions (the teeth should be broken into fragments) should be taken from the animal immediately after death, and placed in a solution of chromic acid 1 part, water 100 parts, nitric acid 2 parts or hydrochloric acid 5 parts, for ten days or two weeks. The bone fragments are then in condition to cut with the razor; or, if it is not desirable to make sections immediately, they may be kept in alcohol until this is desired, and, if they should happen to have become too soft, they become sufficiently hardened by the action of the alcohol. The teeth may require somewhat longer maceration in the acid solution; but eventually they will also be reduced to a similar state fit for manipulation. Thus prepared, the bone-corpuscles, with their radiated prolongations, fill the lacunæ and canaliculi, and may be stained with carmine or hæmatoxylin and mounted, as described, in glycerin or damar varnish. So, too, the dentinal canals or tubules are filled with the soft bioplasmic prolongations of the cells of the tooth-pulp, and may be stained as well. Of course, sections should be

made in different directions in both bone and teeth, in order that the relations of the different elements may be studied.

In this way only can we obtain a proper understanding of the structure of living bone and tooth, the only element which is removed being the lime-salts,—a result which does not affect the morphology of the tissue.

The second method is to take clean dry bone and teeth, and, having properly secured them in a vice, make thin sections by means of a fine saw made of watch-spring. These are then ground down to a proper thinness, either by the aid of water upon a rotary grindstone, or by rubbing with moist emery-powder on a ground-glass plate against which they are pressed by the finger or a piece of cork. They are then polished on a wet hone, carefully washed, and dried, after which they may be mounted in balsam, turpentine, damar, or glycerin, or may even be mounted dry. The contrast between the lacunæ and canaliculi, on the one hand, and the matrix, on the other, is strongest in the latter method, because we have the empty spaces filled with air, a medium refracting very differently from the inorganic matrix. When the spaces are filled with balsam or turpentine,—as can readily be done by soaking the sections in the latter menstruum, and then, if desired, transferring to balsam,—the density of the two elements is approximated, and the spaces appear less dark. A good idea of the relation of the lacunæ and canaliculi to the Haversian canals in the Haversian system of bone is thus obtained, and they should be viewed in transverse and longitudinal sections; but it is plain that we have not here the structure of a living tissue, while the trouble and time required in thus preparing sections are not likely to invite many to pursue this method.

CARTILAGE.

Cartilage is perhaps the easiest to examine of all tissues requiring anything like special preparation. Its consistence, under ordinary circumstances, is exactly what is desirable for making sections, and these are readily stained and mounted; though it must be remembered that cartilage is readily altered by fluid media brought in contact with it. Salt solution, or serum, or glycerin, containing a few drops of acetic acid to the ounce, is suitable. *Hyaline* cartilage may be obtained from the costal cartilage of small animals and children, or from fœtal cartilages; *reticular* or *fibrous* cartilage, in the epiglottis or cartilages of the ear; *connective*-tissue cartilage, in the cartilage of the eyelid. If cartilage has become calcified, its treatment becomes similar to that of bone.

PRESERVING SPECIMENS.

The favorite menstruum at present used for mounting all preparations, except the silver and gold stained tissues, is the *damar lac*. To this, preparations are directly transferred from turpentine or oil of cloves, the excess of these being removed by momentary contact with bibulous paper, covered, and allowed to dry; the excess of damar at the edge of the preparation answering at once for cement and wall of the cell. Preparations which have been previously surrounded by watery solutions or glycerin must of course be washed in alcohol before they can be placed in turpentine or oil of cloves; nor can preparations be directly transferred from alcohol to damar lac, but must first pass through turpentine or oil of cloves.

For preparations previously immersed in aqueous

menstrua, which do not admit of transfer to alcohol, and thence to turpentine or oil of cloves, *glycerin* is used, and the cover cemented with the damar lac. Carmine preparations should be mounted in glycerin containing five drops of acetic acid to the ounce, the coloration being thus more vividly retained through the agency of the acid. *Saturated solution of acetate of potash* is also suitable for preparations requiring aqueous menstrua, and is similar to acetic acid glycerin.

Oil of cloves is also much used for mounting, and is the favorite medium for making sections transparent which are not sufficiently so. For this purpose it is more satisfactory than turpentine. An immersion of from a few seconds to several hours is permitted, and sections may remain in the oil of cloves until mounted in damar.

After considerable experience with a large number of preservatives, I am still inclined to prefer *glycerin* to all others,—at least for stained preparations. When it or any aqueous preservative is used, the thin glass cover requires to be cemented at the edges, and for this nothing is so efficient or neat as the *damar lac*. It should be carefully applied with a fine brush, or solid glass rod, slightly overlapping the edges of the thin glass cover, from which all moisture should be previously removed by bibulous paper.

THE END.

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